Characteristics changes of Chinese bayberry (*Myrica rubra*) during different growth stages

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Abstract The study evaluated the flavor related properties of the Chinese bayberry (*Myrica rubra*) during different growth stages. The weight, total soluble solids, sugar composition and total anthocyanin content were the highest in full-ripe bayberry fruit. Total phenolic content decreased during growth and full-ripe fruit juice showed the lowest antioxidant activities (DPPH, FRAP, and ABTS). Forty-seven volatiles were detected in the different ripening stages of bayberry, and 20 of them were identified as important aroma contributors using GC–MS–O. PCA based on the data of GC–MS and electronic nose allowed to clearly differentiate all the ripening stages. The results also indicated that D-limonene (D3) with “lemon, citrus” note was most closely associated with the unripe bayberry,

nonanal (A5, “citrus, flower” note), decanal (A7, “orange” note), β -ocimene (D5, “mushroom” note), and isocaryophyllene (D8, “wood” note) were associated with the mid-ripe bayberry, and the full-ripe bayberry fruit were characterized by hexanal (A1, “green” note), (*E*)-2-octenal (A4, “green” note), (*E*)-2-nonenal (A6, “cucumber” note), 1-hexanol (B1, “green” note), (*Z*)-3-nonen-1-ol (B3, “cucumber” note), and methyl benzoate (C6, “herb” note).

Keywords Bayberry (*Myrica rubra*) · Flavor · Principal component analysis (PCA) · Ripeness · GC–MS–O · Electronic nose

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Introduction

The Chinese bayberry (*Myrica rubra*) is a famous fruit because of specific taste and high nutrition (Cheng et al. 2015a, b). However, lots of fruits dropped before maturation because it grows in the rainy season. Also farmers have to do some fruit thinning in order to ensure quality. Thus, many bayberries dropping wasted, and should be developed into new resources. There are a great flavor changes on Chinese Bayberry during growth stages. So it is necessary to investigate its characteristics changes in order to reveal the relationship between consumer acceptance and physicochemical properties related to taste evaluation and provide scientific data for industrial practice and development of new products.

Bayberry fruits changes in physicochemical properties are related to taste evaluation during growth stages, such as total soluble solids (TSS), titratable acidity (TA), color, sugars (glucose, fructose, and sucrose), total anthocyanin content (TAC) and total phenolic contents (TPC) during growth stages. Bayberry color changes mainly contributed

by the increase of TAC (Fang et al. 2009). The sweet taste changes may be contributed by the increase of TSS and the decrease of TA (Cheng et al. 2016). There is a significant correlation between antioxidant activity and growth stage (Oliveira et al. 2011). The volatile compounds could also change significantly, however there is no information regarding the aroma and volatile compounds during growth (Chambers and Koppel 2013).

In this study, the physicochemical properties, antioxidant activities (AA), and volatile composition of bayberry were investigated during different growth stages. To obtain the information of characteristics of the volatile compounds responsible for the principal flavor, the method of GC–MS–O and E-nose combined with principal component analysis (PCA) were applied, which had been used for other food (Du et al. 2012; Janzanti and Monteiro 2014; Quintero-Soto et al. 2018).

Materials and methods

Fruit materials

The different maturity bayberry fruits (*Myrica rubra* Sieb. et Zucc. cv. biqi) were collected on 14th May, 27th May and 20th June from farmers in Zhejiang Province of China. The appearance characteristics were showed in Fig. 1. The samples (5 kg for each bayberry ripeness stage) picked were immediately transported to the laboratory and frozen in liquid nitrogen and stored at -80°C until required for analysis. For each ripeness stage, a random sample of 30–40 fresh fruits of uniform size was used and the fruit flesh was ground to a pulp using a commercial blender. Bayberry samples juiced by 400 mesh filter were then analyzed in triplicate for the following chemical properties.

Reagents

A mixture of n-alkanes ($\text{C}_8\text{--C}_{20}$) was used for the retention index (RI) analyses. The RI calculation was carried out according to the manufacturer's instructions (Sigma Chemical Co., St. Louis, MO, USA). The cyclohexanone used as the internal standard was purchased from J&K Chemical Ltd (Shanghai, China). Folin-Ciocalteu reagent was purchased from Shanghai Chemical Reagent Company (China). The sugar standards (glucose, fructose, and sucrose), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS), and 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) were obtained from Sigma (St. Louis, MO, USA).

Physicochemical properties

TSS, TA and pH

TSS of bayberry juice was measured using a digital refractometer (Atago, Tokyo, Japan). The pH and TA of bayberry juice were quantified using a Mettler automatic titrator. The juice sample was diluted with distilled water and titrated with 0.1 mol/L NaOH to a pH of 8.2. The TA was expressed as g of citric acid equivalent per 100 g FW (fresh weight). Fruit maturity index was determined as the ratio TSS/TA.

Sugars (glucose, fructose, sucrose)

The detection of sugars (glucose, fructose, sucrose) was performed by high-performance anion-exchange chromatography with pulsed amperometric detection (ICS-3000, Dionex, Germany). The bayberry juice was filtered through a $0.45\ \mu\text{m}$ membrane filter. The sugars in each sample were identified and quantified using an external

Fig. 1 Radar plots depicting the sensory attributes of different ripening stages of “Bi qi” bayberry samples: the intensities of the respective attributes were rated on a scale of 0 (no perception) to 10 (strong perception) for each sample

standard method with commercial standards (Niponsak et al. 2015).

TAC and TPC

TAC was detected using the pH differential method (Sui et al. 2016) with some modification. In the method, the absorbance of pH = 1.0 and pH = 4.5 buffer-diluted bayberry extract were measured at 510 nm and 700 nm by UV–visible spectrophotometer (UV-2550, SHIMADZU Co., Ltd. Japan). The monomeric anthocyanin was calculated using the molecular weight of cyanidin-3-glucoside (449.2). TPC were estimated using the method described by Fang et al. (2009) with a slight modification. Quantification was based on the calibration curve generated with gallic acid standard solutions, and contents were expressed as mg gallic acid equivalent (GAE)/kg of juice.

Color

Color of bayberry juices were evaluated using the CIELab chroma system (Berenguer et al. 2016). The colorimetric measurements were carried out using a tristimulus colorimeter (Minolta Chroma Meter Measuring Head CR-400 Minolta, New Jersey, USA). The CIE-L a b scale was used. The L*C*H° system was adopted to measure the color in this study (Obenland et al. 2012).

Sensory evaluation

The sensory properties of bayberry juices were evaluated using descriptive sensory analysis using a panel of 12 assessors worked in Wahaha Co., Ltd., (Hangzhou, China), aged from 22 to 35 years. They all had expertise in food sensory evaluation. Approximately 15 mL of juice (frozen bayberry fruit of different ripeness were juiced at the same session after thawing step) was served into odor-free, disposable, covered, transparent 50 mL plastic cups to each panelist along with the questionnaire, one at a time, with about 3 min wait between samples. A complete list of sensory terms and their definitions is shown in Fig. 1. The intensities of the sensory attributes were evaluated using unstructured 10 cm-long lines ranging from “no perception” to “strong perception,” and sensory results were recorded as the distances from the origin in cm, with intermediate steps of 0.5 cm. The sensory attributes intensities were presented as the mean of the scores provided by the 12 panel assessors.

Antioxidant activity

The antioxidant activity of sample was evaluated by three methods: DPPH, FRAP, and ABTS (Huang et al. 2014). The detection of DPPH was done at 517 nm in dark room

at normal temperature. The detection of FRAP and ABTS was done at 593 nm and 734 nm separately. The results were expressed as mg of TEAC (Trolox equivalent antioxidant capacity) per 100 g FW (fresh weight).

Aroma determination

Volatile compounds were extracted by the headspace solid phase microextraction (HS-SPME) method and the extractions were detected by gas chromatography/mass spectrometry (GC/MS) system equipped with a DB-5 capillary column (30 m × 0.25 mm, 0.25 μm film thickness) (Agilent Technologies, Inc., Santa Clara, CA, USA), coupled with a sniffing port (Sniffer 9000, Brechbühler, Switzerland), and also acquired by the portable PEN 2 E-nose (Win Muster Air-sense Analytics Inc., Germany) according to Cheng et al. (2015a, b), sensor arrays and performance in PEN2 electronic nose was showed in Table 1.

Statistical data analysis

One-way analysis of variance and Tukey's tests were performed to identify the differences between the means using SPSS software (version 17.0; SPSS, Inc., Chicago, IL, USA). *p* values < 0.05 were considered statistically significant. The column figures in the context were plotted using Origin software (version 8.5; Northampton, MA, USA), and PCA of the aroma-active volatile compounds was performed using Unscrambler software (version 9.7; CAMO AS, Trondheim, Norway). Data from the characterization of the bayberry samples was reported as mean ± standard deviation for triplicate determinations.

Results and discussion

Changes in physicochemical properties during ripening stages

TA, pH and TSS

The physicochemical composition of bayberry fruit were given in Table 2. TA values ranged from 0.69 to 5.06 g/100 g (citric acid equivalent) among different ripening stages, which corresponded with the differences in pH values (2.52–3.38). Mid-ripe bayberry juice contained the highest TA (5.06 g/100 g) and the lowest pH value (2.52) whereas ripe bayberry juice contained the lowest (0.69 g/100 g) and the highest pH value (3.38). The full-ripe bayberry fruit contained the highest TSS (11 ± 0.1%), while unripe and mid-ripe bayberry contained less, ranging from 4.53 to 5.33 (%). The TSS: acid ratio is often used as a tool to judge fruit taste during fruit development with a

Table 1 Sensor arrays and performance in PEN2 electronic nose

NO.	Sensors	Character	Detection limit
S1	W1C	Sensitive to aromatic compounds	Toluene (10 ppm)
S2	W5S	Sensitive to nitrogen oxides	NO ₂ (1 ppm)
S3	W3C	Sensitive to ammonia and aromatic compounds	Benzene (10 ppm)
S4	W6S	Sensitive to hydrogen	H ₂ (100 ppb)
S5	W5C	Sensitive to alkenes and aromatic compounds	Propane (1 ppm)
S6	W1S	Sensitive to methane broad range	CH ₄ (100 ppm)
S7	W1W	Sensitive to sulphur and terpene compounds	H ₂ S (1 ppm)
S8	W2S	Sensitive to alcohols and partially aromatic compounds	CO (100 ppm)
S9	W2W	Sensitive to aromatic and sulphur organic compounds	H ₂ S (1 ppm)
S10	W3S	Sensitive to alkane	CH ₄ (10 ppm)

Table 2 Physicochemical characterization of bayberry juice during different ripeness

Index ¹	Contents in three ripening stages (mean $\pm$ SD) ²		
	Unripe	Mid-ripe	Full-ripe
Weight	1.06 $\pm$ 0.06 ^{c3}	2.04 $\pm$ 0.04 ^{b3}	7.66 $\pm$ 0.34 ^{a3}
pH	2.91 $\pm$ 0.01 ^b	2.52 $\pm$ 0.01 ^c	3.38 $\pm$ 0.01 ^a
TSS	4.53 $\pm$ 0.21 ^c	5.33 $\pm$ 0.06 ^b	11 $\pm$ 0.1 ^a
TA	3.06 $\pm$ 0.06 ^b	5.06 $\pm$ 0.02 ^a	0.69 $\pm$ 0 ^c
TSS/TA	1.48 $\pm$ 0.09 ^b	1.05 $\pm$ 0.01 ^c	15.86 $\pm$ 0.2 ^a
Glucose	0.08 $\pm$ 0.01 ^b	0.13 $\pm$ 0.01 ^b	2.21 $\pm$ 0.1 ^a
Fructose	0.06 $\pm$ 0 ^b	0.11 $\pm$ 0.01 ^b	2.41 $\pm$ 0.13 ^a
Sucrose	0.17 $\pm$ 0.01 ^b	0.32 $\pm$ 0.02 ^b	7.65 $\pm$ 0.34 ^a
Sugar	0.31 $\pm$ 0.02 ^b	0.57 $\pm$ 0.04 ^b	12.27 $\pm$ 0.57 ^a
TPC	1452.26 $\pm$ 1.64 ^a	961.2 $\pm$ 0.62 ^b	713.6 $\pm$ 0.62 ^c
TAC	nd	nd	217.7 $\pm$ 0.35 ^a
L	60.08 $\pm$ 1.88 ^a	58.46 $\pm$ 1.06 ^a	26.99 $\pm$ 0.72 ^b
a	8.84 $\pm$ 0.09 ^b	4.31 $\pm$ 0.18 ^c	11.5 $\pm$ 0.47 ^a
b	4.49 $\pm$ 0.29 ^b	8.11 $\pm$ 0.43 ^a	3.05 $\pm$ 0.14 ^c
C*	9.92 $\pm$ 0.2 ^b	9.18 $\pm$ 0.46 ^b	11.9 $\pm$ 0.49 ^a
h _{ab}	0.47 $\pm$ 0.02 ^b	1.08 $\pm$ 0.01 ^a	0.26 $\pm$ 0 ^c

¹The units for each index were as following: Weight (g), TA (titratable acidity, g/100 g), TSS (Total soluble solids, %), sugar (glucose, fructose, and sucrose, g/100 g), TAC (total anthocyanin content, mg GAE/kg), TPC (total phenolic contents, mg GAE/kg)

²The mean and standard deviation (n = 3) were calculated for three replicates, data listed were the mean of three assays $\pm$ SD (standard deviation)

³Values in subtotal data with different letters (a, b and c) are significantly different ($p < 0.05$)

⁴nd: The contents of TAC were not detected in bayberry juices

high ratio corresponding to a cultivar that can be classified as sweet taste. Full-ripe bayberry fruit contained significantly higher ratio with the sweetest taste.

Composition of soluble sugars

The results showed the total amounts of soluble sugars ranged from 0.31 to 12.27 g/100 g fresh weight among

different ripening stages. Among the soluble sugars detected in bayberry fruits, sucrose was the most abundant, followed by glucose and then fructose. The full-ripe bayberry fruit contained the highest sucrose, as well as the highest glucose and fructose. The higher sugars levels could contribute to the stronger “sweet” attribute for the full-ripe bayberry fruit.

TAC, TPC and color

According to the results showed in Table 2, the anthocyanins (217.7 $\pm$ 0.35 mg/kg) were only detected in the full-ripe bayberry. The color of bayberry change from yellow-green to red gradually during different growth stages, which was also reflected by L*, C*, and H° values. The unripe and mid-ripe bayberry had higher lightness (L*) values and the ripe had lower. With the content of chlorophyll decreased and the content of anthocyanins increased, the pH and TA varied during bayberry growth, the color of yellow (mixed color of red and green in juice) become obvious in bayberry juice, which may led to lower a* values and higher b values in the mid-ripe bayberry (Harborne, 1995). The full-ripe bayberry, which presented as red colors, had the highest chroma values (a and C*). The differences among different ripe stages were contributed by the anthocyanin composition and content as well as other photochemical in the bayberry fruits.

TPC decreased with bayberry growth (Table 2), ranging from 1452.26 $\pm$ 1.64 to 713.6 $\pm$ 0.62 GAE mg/kg. It might contributed stronger “astringent” flavor to the unripe and mid-ripe bayberry (Laaksonen 2011). Interactions between polyphenols with proteins had been used to predict perceived astringency (Llaudy et al. 2004; Monteleone et al. 2004)

Sensory characteristics

The sensory characteristics of bayberry fruit were showed in Fig. 1. “Green”, “sweet”, “sour”, and “astringent”

notes were found in all of the bayberry ripening stages. Sensory panelists reported that the quality of the fruit increased from unripe to full-ripe, which was associated with a lesser degree of “sour” and “astringent” and increased levels of “sweet” and “fruity”. The different intensities of “sour” and “astringent” of the fruit might be due to the change of the TA value and TPC content, while “sweet” of the fruit might be due to TSS that was showed in Table 2 (Yu et al. 2018). The TPC in bayberry fruit were consisting of gallic acid, myricetin glycosides, quercetin glycosides and kaempferol glycosides (Yang 2007). Gallic acid has bitterness and astringency (Robichaud and Noble 1990). Flavonoid glycoside may also be the main compound of astringency (Scharbert and Hofmann 2005; Scharbert et al. 2004). Higher intensities of the “fruity”, “sweet”, and “juicy” attributes were perceived in the full-ripe stage. The quality of the fruit was also affected by TAC (that made the bayberry red) and aroma compounds.

Antioxidant ability of bayberry with different ripening stages

Based on the DPPH, FRAP and ABTS assays, the bayberry juices during different ripening stages had considerable capacities of antioxidant activities (Fig. 2). Among the three bayberry juices, ripe bayberry had the lowest TEAC values for all assay methods. The antioxidant ability of unripe and mid-ripe bayberry were similar in DPPH and ABTS assays. In FRAP assays, unripe bayberry had the highest TEAC values for FRAP (433.45 ± 37.61 TEAC mg/100 g). TPC showed a similar evolution pattern: while unripe, mid-ripe and full-ripe fruits displayed significantly different values (Table 2). Antioxidant activity of bayberry was highly correlated with the contents of phenolics, including flavonoids (Chen et al. 2016).

Fig. 2 The antioxidant activities (DPPH, FRAP, and ABTS) of different ripening stages of bayberry fruit

Changes of aroma compounds during ripening stages

A total of 47 compounds were identified from different ripening stages of bayberry fruits by using HS-SPME and GC–MS (Table 3), including 8 aldehydes (A1–A8), 4 alcohols (B1–B4), 11 esters (C1–C11), 11 terpenes (D1–D11) and 13 others (E1–E13) (Table 3). The unripe and mid-ripe fruits contain a similar number of volatiles (19 and 17 identified compounds, respectively), while full-ripe fruits presented the highest number of volatiles (37). The volatile compounds identified in this study were also reported in other studies concerning bayberry fruit (Cheng et al. 2015a, b; Kang et al. 2012; Xu et al. 2014).

Aldehydes represent the second major group of volatile compounds. Eight aldehydes were identified, hexanal and nonanal being the most abundant. Aldehydes reach the highest amount at full-ripe maturation. Hexanal (A1) showed a significant increase from unripe (2.08 µg/g) to full-ripe fruits (24.54 µg/g). Hexanal is the most abundant aldehyde and contribute to the “green” odor in full-ripe bayberry fruit by GC–O analysis. However, Janzanti and Monteiro (2014) found that hexanal decreased in passion fruit during ripeness and the reduction of hexanal could be related to the appearing of hexanol. The different change may be caused by the different fruits. Nonanal (A5) with “citrus, flower” note is dominant aldehyde in unripe and mid-ripe bayberry fruit.

Four alcohols were found in the full-ripe bayberry fruit, the highest concentration of alcohols was found in the full-ripe bayberry fruit (14.45 µg/g), with 1-hexanol (B1, 7.16 µg/g) as the main aroma contributor (“flower” and “green”).

Esters are important volatiles of full-ripe bayberry fruit and contribute to the strong fruity and floral odor. The total concentration of esters in full-ripe bayberry fruit was greater than unripe and mid-ripe bayberry fruit. The eleven esters were identified in the full-ripe fruit (11.81 µg/g). Among the esters, methyl benzoate (C6, “herb” note) was the dominant ester in full-ripe bayberry fruit.

A total of 11 terpenes were identified and accounted for the largest proportion of the total volatiles detected in the different ripening bayberry juices. There were significant differences in the terpene concentrations among the ripening stages. Terpenes was reported as the most abundant compounds among the different ripening bayberry fruit (Kang et al. 2012). The β-caryophyllene (D9) was determined as the main terpene (maximum of 1729.88 µg/g) in the mid-ripe bayberry fruit, followed by D-limonene (D3, maximum of 115.9 µg/g) in the unripe bayberry fruit. Six odors were detected by the panelists, indicating that they actively contributed to the aroma profiles of the bayberry fruits. The “lemon, citrus” odor might be due to the

Table 3 Volatile compounds of the bayberry juice in different states of ripeness by GC–MS

Code	RI ¹	Compound	Aroma descriptors ²	Concentration/ $\mu\text{g/g}$ (mean $\pm$ SD) ³		
				Unripe	Mid-ripe	Ripe
<i>Aldehydes</i>						
A1	< 800	Hexanal	Grass, green	2.08 $\pm$ 0.47	0.93 $\pm$ 0.4	24.54 $\pm$ 2
A2	858	(E)-2-Hexenal	Green, apple	nd	nd	1.42 $\pm$ 0.16
A3	1011	Octanal	Lemon, green	0.53 $\pm$ 0.18	1.41 $\pm$ 0.13	1.3 $\pm$ 0.2
A4	1061	(E)-2-Octenal	Green, nut	nd	nd	1.62 $\pm$ 0.6
A5	1113	Nonanal	Citrus, flower	8.31 $\pm$ 4.06	24.45 $\pm$ 8.12	6.97 $\pm$ 0.27
A6	1169	(E)-2-Nonenal	Cucumber	nd	nd	10.89 $\pm$ 1.14
A7	1213	Decanal	Soap, orange	2.93 $\pm$ 1.96	6.46 $\pm$ 2.03	1.95 $\pm$ 0.06
A8	1218	2,4-Nonadienal		nd	nd	0.3 $\pm$ 0.06
Subtotal ⁴				13.85 $\pm$ 6.14 ^c	33.24 $\pm$ 10.45 ^b	48.99 $\pm$ 3.06 ^a
<i>Alcohols</i>						
B1	876.5	1-Hexanol	Flower, green	nd	nd	7.16 $\pm$ 1.02
B2	1070	1-Octanol		nd	nd	0.91 $\pm$ 0.15
B3	1163	(Z)-3-Nonen-1-ol	Cucumber	nd	nd	4.93 $\pm$ 0.48
B4	1172	1-Nonanol		nd	nd	1.45 $\pm$ 0.32
Subtotal				nd ^b	nd ^b	14.45 $\pm$ 1.02 ^a
<i>Esters</i>						
C1	< 800	Methyl acetate	Ether, sweet	nd	nd	0.92 $\pm$ 0.03
C2	< 800	Ethyl acetate	Pineapple	nd	nd	0.69 $\pm$ 0.08
C3	933.2	Methyl hexanoate	Fruit, sweet	nd	nd	1.04 $\pm$ 0.1
C4	932	Methyl (Z)-3-hexenoate		nd	nd	0.51 $\pm$ 0.02
C5	999	Ethyl hexanoate		nd	nd	0.73 $\pm$ 0.15
C6	1103	Methyl benzoate	Herb, prune	nd	nd	3.26 $\pm$ 0.68
C7	1177	Ethyl benzoate		nd	nd	1.14 $\pm$ 0.28
C8	1197	Ethyl caprylate		nd	nd	0.9 $\pm$ 0.29
C9	1222	Methyl 3-nonenoate	Oil, fat	nd	nd	1.57 $\pm$ 0.33
C10	1232	Methyl 2-nonenoate		nd	nd	0.85 $\pm$ 0.3
C11	1794	Ethyl tetradecanoate		nd	nd	0.2 $\pm$ 0.02
Subtotal				nd ^b	nd ^b	11.81 $\pm$ 1.79 ^a
<i>Terpenes</i>						
D1	940	α -Pinene	Pine	nd	nd	0.58 $\pm$ 0.19
D2	1009	β -Phellandrene		1.45 $\pm$ 0.35	nd	nd
D3	1030	D-limonene	Lemon, citrus	115.95 $\pm$ 7.9	26.8 $\pm$ 4.92	nd
D4	1040	trans- β -Ocimene		nd	1.56 $\pm$ 0.45	nd
D5	1051	β -Ocimene	Herb, mushroom	4.92 $\pm$ 1.32	7.79 $\pm$ 2.77	0.81 $\pm$ 0.26
D6	1066	γ -Terpinene		5.47 $\pm$ 0.54	1.79 $\pm$ 0.49	nd
D7	1109	Linalool	Flower	5 $\pm$ 0.36	6.74 $\pm$ 0.98	7.53 $\pm$ 1.38
D8	1409	Isocaryophyllene	Wood	4.5 $\pm$ 2.38	8.22 $\pm$ 1.46	0.94 $\pm$ 0.1
D9	1430	β -Caryophyllene	Wood, spice	1321.31 $\pm$ 183.73	1729.88 $\pm$ 288.21	48.49 $\pm$ 15.57
D10	1607	Caryophyllene oxide		28.17 $\pm$ 5.87	36.98 $\pm$ 1.45	1.84 $\pm$ 0.63
D11	1681	Isoaromadendrene epoxide		nd	nd	4.85 $\pm$ 0.49
Subtotal				1486.77 $\pm$ 201.08 ^a	1819.74 $\pm$ 294.1 ^a	65.05 $\pm$ 18.43 ^b
<i>Others</i>						
E1	< 800	Benzene		nd	nd	0.38 $\pm$ 0.02
E2	887	P-Xylene		1 $\pm$ 0.16	1.17 $\pm$ 0.25	0.66 $\pm$ 0.06
E3	1001	3-Octanone		nd	nd	0.25 $\pm$ 0.01
E4	1014	2-Pentyl-Furan		nd	nd	1.36 $\pm$ 0.29

Table 3 continued

Code	RI ¹	Compound	Aroma descriptors ²	Concentration/ $\mu\text{g/g}$ (mean $\pm$ SD) ³		
				Unripe	Mid-ripe	Ripe
E5	1028	O-cymene		1nd.45	5.19 $\pm$ 0.27	nd
E6	1099	3-Heptene		nd	nd	6.95 $\pm$ 0.64
E7	1123	(E)-1-butoxy-2-Hexene		1.66 $\pm$ 0.16	nd	nd
E8	1140	Pentyl-cyclohexane		7.7 $\pm$ 0.83	6.06 $\pm$ 1.82	nd
E9	1173	D-carvone		1.68 $\pm$ 0.86	nd	nd
E10	1374	Dihydro-5-pentyl-2(3H)-Furanone	Sweet, spice	nd	nd	0.52 $\pm$ 0.06
E11	1488	Z,Z,Z-1,5,9,9-tetramethyl-1,4,7,-Cycloundecatriene		54.15 $\pm$ 1.78	77.32 $\pm$ 13.14	2.63 $\pm$ 0.9
E12	1530	4-Methyl-3-cyclohexene-1-carboxaldehyde		nd	40.13 $\pm$ 8.7	nd
E13	1602	2,6-Diethyl-pyridine		9.72 $\pm$ 5.19	nd	nd
Subtotal				85.92 $\pm$ 4.53 ^b	129.88 $\pm$ 23.04 ^a	12.76 $\pm$ 1.87 ^c

¹RI: retention indices²Odor description at GC-sniffing port³ $\mu\text{g/g}$: concentration was expressed in microgram per gram of juice, cyclohexanone as internal standard, and data listed were the mean of three assays $\pm$ SD (standard deviation)⁴Values in subtotal data with different letters (a, b and c) are significantly different ($p < 0.05$)

presence of D-limonene (D3), which was the most important aroma-active compound found in the unripe bayberry fruit. Further, the “mushroom” odor might be due to the presence of β -ocimene (D5) detected by GC–MS, and was perceived in all the ripening stages. The β -caryophyllene (D9) with “woody” odor was the predominant compound and it also could contribute to the final sensory evaluation of bayberry cultivars (Cheng et al. 2015a, b).

Principal component analysis of aroma-active compounds

PCA was applied to the aroma data and differentiated three different ripening stages according to their unique aroma-active compounds (Table 3). The compounds detected can

be grouped and related statistically to each ripening stage as shown in Fig. 3. And the findings showed a good agreement with the results obtained by electronic nose (supplementary 1). The unripe bayberry fruit was loaded positively on PC1 and PC2. The bayberry fruit from the mid-ripe stage of ripeness was loaded positively on PC1 and negatively on PC2, and the full-ripe bayberry fruit was loaded negatively on PC1 and PC2. D-Limonene (D3) with “lemon, citrus” note was loaded near to the unripe bayberry fruit, indicating it was higher in the unripe bayberry and most closely associated with the unripe bayberry, thus characterizing the unripe bayberry fruit. The mid-ripe bayberry fruit showed higher level of nonanal (A5, “citrus, flower” note), decanal (A7, “orange” note), β -ocimene (D5, “mushroom” note), and isocaryophyllene (D8,

Fig. 3 PCA plots of the aroma-active compounds identified in bayberry fruit during different ripening stages

“wood” note). The β -caryophyllene (D9, “wood” note) showed similar level in the unripe and mid-ripe stage of ripeness, characterizing both unripe and mid-ripe bayberry fruit. The full-ripe bayberry fruit were characterized by higher levels of hexanal (A1, “green” note), (*E*)-2-octenal (A4, “green” note), (*E*)-2-nonenal (A6, “cucumber” note), 1-hexanol (B1, “green” note), (*Z*)-3-nonen-1-ol (B3, “cucumber” note), and methyl benzoate (C6, “herb” note).

Conclusion

In the present study, the effect of ripening stages on the changes of physicochemical properties, antioxidant activities, and aroma of bayberry were evaluated. The weight, TSS, sugars and total anthocyanin content increased significantly during ripening. While total phenolic contents (TPC) decreased across the same period and full-ripe fruit showed the lowest antioxidant activities (DPPH, FRAP, and ABTS). Thus, the antioxidant activities of ripe juice would increase if the unripe fruit juice was used. Twenty of detected volatiles were identified as aroma contributors for the bayberry fruit using GC–MS–O. Preliminary results confirm the usefulness of GC–MS and E-nose for classification and future quality control of bayberry. This combination for the GC–MS analysis with chemometric methods can be applied to discriminate the bayberry samples from different stages successfully. Furthermore, the different flavors of different ripen stages would increase the bayberry products with special taste and aroma.

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